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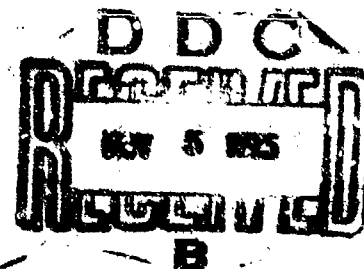
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**RADIATION-RELEASED HISTAMINE  
IN THE RHESUS MONKEY AS MODIFIED  
BY MAST CELL DEPLETION  
AND ANTIHISTAMINE**

T. F. Doyle

T. A. Strike



**ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE**  
**Defense Nuclear Agency**  
**Bethesda, Maryland**

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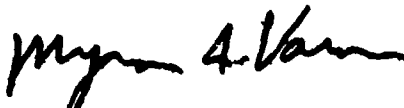
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T. F. DOYLE  
T. A. STRIKE

  
D. O. CARPENTER  
Chairman  
Neurobiology Department

  
MYRON I. VARON  
Captain MC USN  
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE  
Defense Nuclear Agency  
Bethesda, Maryland

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FOREWORD  
(Nontechnical summary)

Circulating blood histamine levels of rhesus monkeys exposed to 4000 rads of ionizing radiation were measured after histamine catabolism was blocked with aminoguanidine (10 mg/kg). Histamine levels increased from  $26 \pm 13.5$  to  $235 \pm 16$  ng/ml at 3 min postirradiation. When monkeys were pretreated with an  $H_1$  antagonist (chlorpheniramine, 3 mg/kg) the circulating blood histamine levels increased from  $25.7 \pm 13.5$  to  $462 \pm 226$  ng/ml. When mast cell histamine was depleted by four consecutive daily injections of compound 48/80 (1 mg/kg per day), no postirradiation increase in histamine concentration was measured. When monkeys were given 48/80 (1 mg/kg) 20 min after a 4000-rad dose of ionizing radiation, histamine concentration increased from  $18 \pm 2$  to  $35 \pm 9$  ng/ml.

These studies show that a 4000-rad dose of ionizing radiation releases a significant amount of mast cell histamine, an amount which, when compared to similar amounts of exogenous histamine, is sufficient to cause the hypotension and other effects seen after this dose of radiation. The mast cell histamine was almost all released by a 4000-rad dose of radiation judged by the failure of 48/80 to release large amounts of histamine when given 20 min after irradiation.

## ABSTRACT

Changes in blood histamine concentrations of rhesus monkeys were measured after a 4000-rad dose of mixed gamma-neutron radiation. All animals were pretreated with aminoguanidine to retard histamine catabolism. Histamine concentrations increased from  $26 \pm 13.5$  to  $235 \pm 16$  ng/ml after irradiation. When the animals were pretreated with an antihistamine, chlorpheniramine (3 mg/kg), histamine concentrations changed from  $25.7 \pm 13.5$  to  $462 \pm 226$  ng/ml after irradiation. When the monkeys were pretreated with a specific mast cell histamine depleter, compound 48/80 (1 mg/kg per day) for four consecutive days and then irradiated (4000 rads), histamine concentrations did not change significantly. When 48/80 was given 20 min after irradiation, histamine concentrations changed from  $18 \pm 2$  ng/ml to a maximum of  $35 \pm 9$  ng/ml after 48/80 injection.

## I. INTRODUCTION

Increased blood histamine levels following ionizing radiation have been reported in rats and man.<sup>2,7,8</sup> These increased histamine levels appear to correlate well with hypotension<sup>8</sup> observed postirradiation as well as a reduction in the number of tissue mast cells.<sup>7</sup> Because ionizing radiation does cause disruption of mast cells, releasing the cellular contents, the suggestion has been made that mast cell histamine release could be responsible for the observed hypotension.<sup>8</sup>

These experiments have been designed to show (1) the amount of histamine released by 4000 rads of ionizing radiation, (2) the amount of histamine which is blocked from receptor sites by an antihistamine, (3) whether the released histamine is of mast cell or nonmast cell origin and (4) whether the histamine released could be responsible for the observed hypotension.

## II. MATERIALS AND METHODS

Healthy monkeys (Macaca mulatta) of both sexes, 2-3 years of age and weighing 3-4 kg, were used in these experiments. Each monkey was anesthetized with sodium pentobarbital\* (50 mg), and catheters were inserted into a femoral artery and vein. The monkeys were allowed to recover from the surgery and then were placed in restraining chairs where they remained until the completion of the experiment.

Few data are available on the histamine measurements of old-world monkeys because of the presence in these animals of high concentrations of histaminase which rapidly catabolizes free circulating histamine.<sup>4</sup> Therefore, all histamine measurements

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\* Nembutal, Abbott Laboratories, North Chicago, Illinois

were made only after the animals were treated with aminoguanidine, \* a histaminase inhibitor.<sup>5</sup>

Discrimination between mast cell and nonmast cell histamine was accomplished using a specific mast cell histamine releaser, compound 48/80,<sup>†3</sup> which has no direct effect on levels of nonmast cell histamine. Treatment with compound 48/80 was continued until only minimal increases in blood histamine were noted, indicating almost complete mast cell histamine release.

The following experiments were conducted:

Experiment 1. Two monkeys were given aminoguanidine (10 mg/kg) intravenously to retard histamine catabolism and 30 minutes later received a single, whole-body 4000-rad dose of mixed gamma-neutron radiation. Blood pressure was recorded continuously using a pressure transducer and polygraph attached to the femoral arterial catheter. Arterial blood samples were taken at intervals for histamine determinations.

Experiment 2. Four monkeys were treated the same as those of Experiment 1, but in addition received an H<sub>1</sub> antagonist, chlorpheniramine<sup>‡</sup> (3 mg/kg), 30 minutes before irradiation.

Experiment 3. Seven monkeys were given aminoguanidine and 30 minutes later the histamine-liberator compound 48/80 (1 mg/kg) was injected intravenously. Blood pressure was monitored before and for 10 minutes after injection of compound 48/80. Preinjection and 2-min postinjection blood samples were taken for histamine

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\* J. T. Baker Chemical Company, Phillipsburg, New Jersey

† Burroughs Wellcome Company, Tuckahoe, New York

‡ Chlor-Trimeton. Schering Corporation, Bloomfield, New Jersey

determinations. This experimental sequence was repeated daily for four consecutive days.

Experiment 4. Four monkeys from Experiment 3 were irradiated with a 4000-rad dose of mixed gamma-neutron radiation approximately 30 minutes after the fourth daily treatment with compound 48/80. Blood samples were taken for histamine determinations at selected times during this period.

Experiment 5. Four monkeys were given a 4000-rad dose of mixed gamma-neutron radiation. Twenty minutes later, aminoguanidine and compound 48/80 were administered. Blood samples were taken for histamine determination before and after drug injection. When the blood sampling was performed in the reactor exposure room, a remotely operated collecting device monitored via closed circuit television was used. Each monkey received heparin (1000 USP units) approximately 30 min before sampling to prevent blood clotting. Each monkey was given unilateral, dorsal-ventral, whole-body irradiation. The mixed gamma-neutron radiation was delivered as a short duration pulse (23-msec width at half-maximum height), and the resulting midline tissue dose was  $4000 \pm 400$  rads.

Student's "t" test was used to determine the statistical significance of the difference between the means of histamine values before and after drug treatment or irradiation. Probability values of less than 0.05 were considered significant.

Histamine was assayed fluorometrically using the procedure of Shore et al.<sup>6</sup> This method involved the extraction of histamine from hemolyzed whole blood into n-butanol. The histamine was then condensed with O-phthalaldehyde to yield a product

with a strong and stable fluorescence. This product was activated at 360 nm and measured at 450 nm.

### III. RESULTS AND DISCUSSION

Figure 1 shows the significant increase in blood histamine which occurred following a 4000-rad dose of ionizing radiation in animals receiving no treatment. When the animals were pretreated with chlorpheniramine, the histamine concentration after irradiation was further increased. Chlorpheniramine is an  $H_1$  antagonist, and the unblocked  $H_2$  receptors when stimulated can still produce a depressor effect. The difference in histamine concentration seen after treatment with chlorpheniramine would thus indicate the degree of  $H_1$  receptor block by chlorpheniramine.

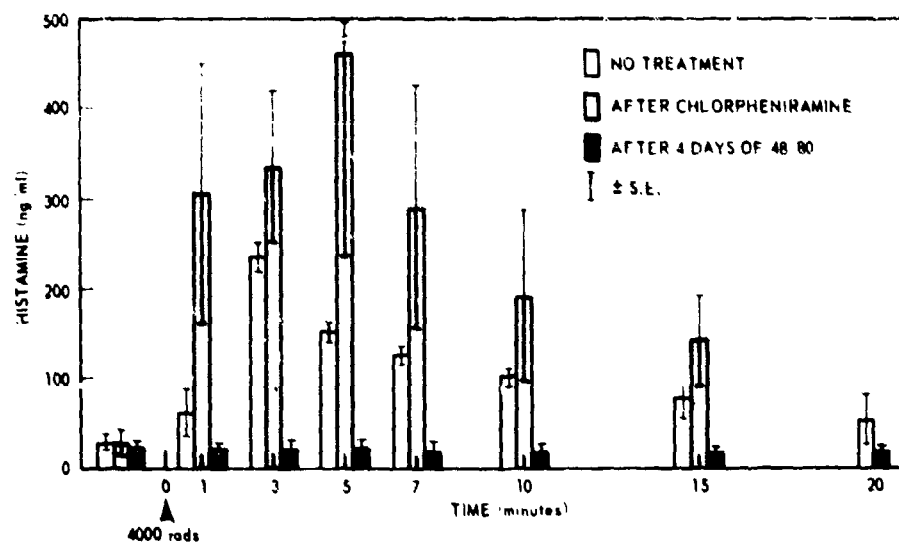


Figure 1. Changes in blood histamine concentration following 4000 rads of ionizing radiation of two untreated monkeys, four monkeys given chlorpheniramine (3 mg/kg) 30 minutes before irradiation and four monkeys treated with 48/80 (1 mg/kg per day) for four consecutive days

Table I shows the percent increase of histamine concentration in animals treated for four consecutive days with compound 48/80, which releases histamine from mast cells. Histamine was measured 2 min after each dose of 48/80.

Table I. Histamine Concentration (ng/ml) and Percent Increase Measured Before and 2 Min After Each Injection of 48/80 (1 mg/kg) Given on Four Consecutive Days

Accumulative dose (mg/kg)	Histamine concentration		Percent increase
	Before 48/80	After 48/80	
1	8.4 ± 1.4	31.8 ± 14	294
2	11.7 ± 2.4	20.3 ± 6.5	73.5
3	10.7 ± 1.5	13.8 ± 2	29
4	10 ± 1.3	11.7 ± 2.3	17

A marked fall in blood pressure occurred after the first dose of 48/80. A smaller decrease occurred after the second dose, and the blood pressure appeared to stabilize after the third dose of 48/80 (Figure 2). This pseudotachyphylaxis is due to depletion of histamine stores rather than to the animals' accommodating to 48/80.

Thirty minutes after the fourth dose of 48/80, the animals were irradiated with 4000 rads of ionizing radiation (Figure 1). No significant increase in blood histamine could be measured after irradiation.

The moderate amount of histamine released when monkeys are irradiated without pretreatment (Figure 1) compared to the negligible amount released when monkeys are given 48/80 (1 mg/kg) after irradiation shows that a 4000-rad dose of irradiation releases most, but not all, of the mast cell histamine (Figure 3). When the  $H_1$

receptors were blocked by chlorpheniramine, the histamine concentration was greatly increased (Figure 1).

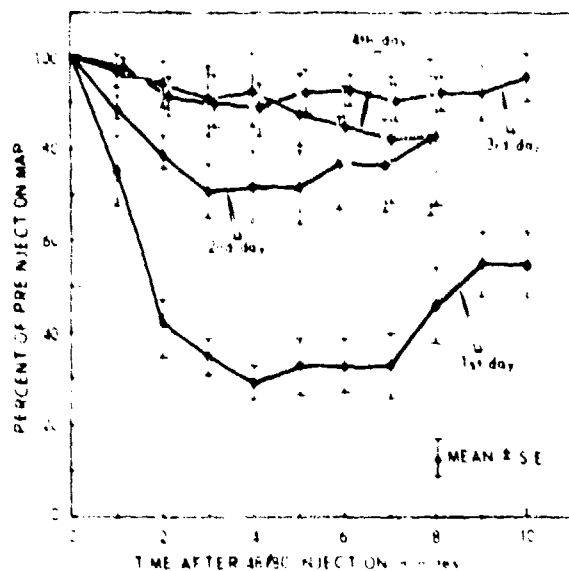


Figure 2.  
Mean arterial pressure changes after injection of compound 48/80 (1 mg/kg) into seven monkeys on four consecutive days

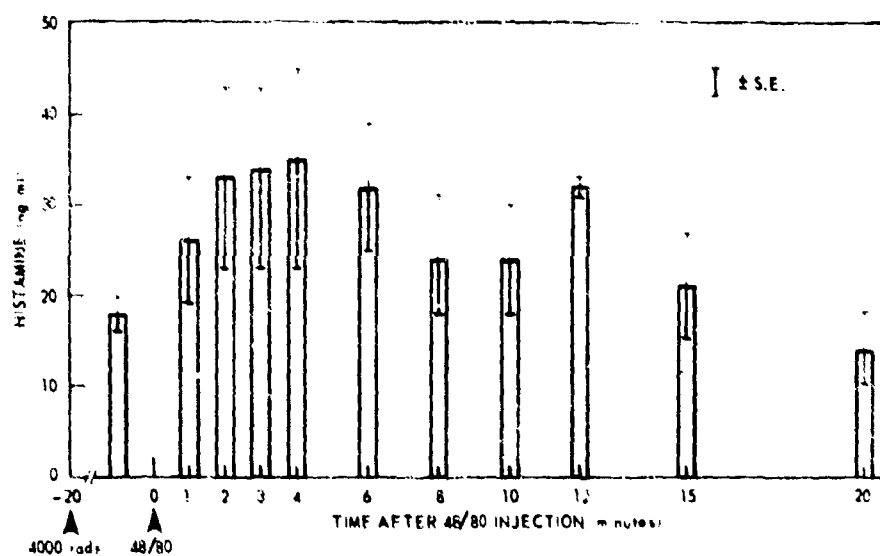


Figure 3. Blood histamine concentration of four monkeys given 48/80 (1 mg/kg) 20 min after a 4000-rad dose of ionizing radiation

#### IV. CONCLUSIONS

Based on circulating histamine concentrations, the following conclusions can be made: (1) a 4000-rad dose of radiation releases a significant amount of histamine; enough, when compared to the effects of exogenous histamine of roughly the same concentration in blood,<sup>1</sup> to produce many of the immediate effects seen after this dose of radiation; (2) the H<sub>1</sub> antagonist chlorpheniramine blocks the attachment to receptors of a significant amount of histamine; (3) most of the histamine released by the 4000 rads of radiation is of mast cell origin; those monkeys depleted of mast cell histamine prior to irradiation showed no increase in histamine concentration following irradiation; and (4) a 4000-rad dose of radiation releases most of the mast cell histamine, 48/80 given 20 min after irradiation produces only a slight increase (17 ng/ml above control value) in circulating histamine concentrations.

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